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Disease Notes

First Report of Lasiodiplodia theobromae Causing Inflorescence Blight and Fruit Rot of Longan (Dimocarpus longan L.) in Puerto Rico

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Dimocarpus longan L., commonly known as longan, is a tropical fruit tree of the Sapindaceae family. From 2008 to 2010, a disease survey for longan was conducted in March and October in Puerto Rico. Fruit rot and inflorescence blight (rotting of the rachis, rachilla, and flowers) were observed in fields of longan at the USDA-ARS Research Farm in Isabela, and two commercial orchards in Puerto Rico. Tissue sections (1 mm²) of diseased inflorescences and surface of the fruit were disinfested with 70% ethanol, rinsed with sterile water, and transferred to acidified potato dextrose agar (APDA). Three isolates of Lasiodiplodia theobromae (Pat.) Griffon & Maubl. (Lt) were isolated from symptomatic tissue and identified morpho-molecularly using a taxonomic key for the Botryosphaeriaceae and DNA sequence analysis (1). In APDA, colonies of Lt had initial greenish-gray aerial mycelia that turned dark brown with age. Pycnidia were dark brown to black. Immature conidia were sub-ovoid to ellipsoid, apex rounded, truncate at the base, thick-walled, hyaline, and one-celled, becoming dark brown, two-celled, and with irregular longitudinal striations when mature. Conidia (n = 50) for all the isolates averaged 26.9 μ m long by 13 μ m wide. For molecular identification, the ITS1-5.8S-ITS2 region and fragments of the β-tubulin and elongation factor 1-alpha (EF1-a) genes were sequenced and BLASTn searches done in GenBank. Accession numbers of gene sequences of Lt submitted to GenBank were KC964546, KC964547, and KC964548 for ITS region; KC964549, KC964550, and KC964551 for β-tubulin; and KC964552, KC964553, and KC964554 for EF1-a. For all genes used, sequences were 99 to 100% identical to reference isolate CBS164.96 of Lt reported in GenBank (accessions AY640255, EU673110, and AY640258). Pathogenicity tests were conducted on six random healthy non-detached inflorescences of longan and six healthy detached fruits per isolate. Unwounded inflorescences and fruit were inoculated with 5-mm

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mycelial disks from 8-day-old pure cultures grown in APDA. Inflorescences were enclosed in plastic bags for 5 days under field conditions while fruits were kept in a humid chamber using plastic boxes for 5 days under laboratory conditions of 25°C and 12 h of fluorescent light. Untreated controls were inoculated with APDA disks only. The experiment was repeated once. Five days after inoculation, isolates of Lt caused inflorescence blight, fruit rot, and aril (flesh) rot. Inflorescences turned brown and flower mummification was observed on the inflorescences. The exocarp (peel) and endocarp (aril) turned dark brown and mycelial growth and pycnidia of Lt were observed on fruits. Untreated controls did not show any symptoms and no fungi were re-isolated from tissue. In diseased inflorescences and fruits, Lt was re-isolated from diseased tissue and identified using morphological and molecular parameters, thus fulfilling Koch's postulates. Lt has been reported to cause dieback, stem end rot, and fruit rot on a wide range of plants host (2,4). In longan, Lt has been reported causing fruit rot in Thailand (3). To our knowledge, this is the first time that Lt has been reported causing inflorescence blight in longan and the first report of Lt causing fruit rot in Puerto Rico.

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